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METHOD DEVELOPMENT AND VALIDATION OF BAMBUTEROL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM BY USING UV SPECTROSCOPY

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ABSTRACT :

Bambuterol Hydrochloride (BAM) is a bronchodilator used to treat and prevent respiratory disorders like asthma or exercise induced bronchospasm and chronic obstructive pulmonary disease (COPD). A new, simple, accurate, precise and economic analytical method for simultaneous estimation of BAM in tablet dosage form has been developed. A survey of literature reveals that Bambuterol Hydrochloride is estimated by UV-Spectrophotometry for this dosage form. The suitable solvent i.e. water is selected according to the solubility of drugs. Simultaneous equation method based on measurement of highest peak wavelength between the range 264nm and then the absorbance of two drugs i.e. BAM is checked at respective wavelength. Then the present UV-Spectrophotometric method was validated as per ICH guidelines.

Keywords: Bambuterol hydrochloride (BAM), Purified Water, UV, Validation

INTRODUCTION :

Analytical Chemistry is a branch of practical chemistry which involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. Pharmaceutical analysis is a branch of science that deals with the analytical procedures used to determine the purity safety and quality of drugs and chemicals. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form.

Types:

Analytical chemistry is the science of making quantitative and qualitative measurements. There are two main types of chemical analysis-

1. Qualitative(Identification)
2. Quantitative(estimation)

1. **Qualitative analysis** is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in sample or not.
2. **Quantitative analytical** techniques are mainly used to quantify any substance or compound in sample.

UV-SPECTROSCOPY:

The principle is based on the measurement of spectrum of a sample containing atoms/molecules. It mainly involves identification of compound on the basis of absorption. Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency or wavelength. Spectrometer is an instrument design to measure the spectrum of a compound. UV wavelengths cover a range approximately from 200 to 400 nm.

- **UV-Spectroscopy based on following law's-**

1. Beer's Law:
   When a beam of monochromatic light is passed through a medium ,absorbance is directly proportional to the concentration of sample.
   FORMULA = A α C
2. Lambert's Law:

When a beam of monochromatic light is passed through a medium ,absorbance is directly proportional to the path length or thickness.

FORMULA = A α L

Beer-Lambert's Law

A=ΣCL

Where,

A=Absorbance

Σ=Molar absorption coefficient C= Concentration of solution L= Path length or thickness

BAMBUTEROL HYDROCHLORIDE:

Chemical Structure:

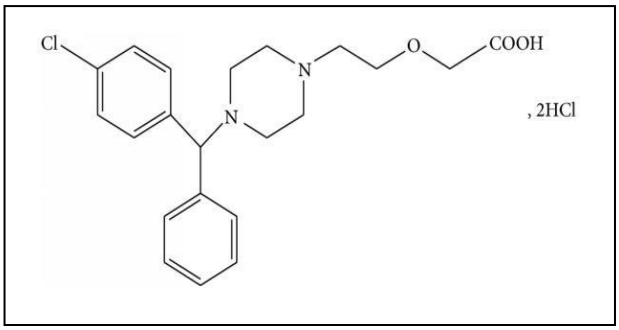

Fig.3: Chemical Structure of Bambuterol Hydrochloride

Chemical name :(RS)-5-[2-(tert-butylamino)-1-hydroxyethyl]benzene-1,3-diylbis(dimethylcarbamate)

Molecular Formula: C18H29N3O5.HCl

Molecular weight: 403.91 gm/mole

Description: A white or almost white crystalline powder.

Solubility: Freely Soluble in water

Category: Beta adrenoceptor agonist

Mechanism of action: Bambuterol is a long acting beta 2 adrenoceptor agonist used in the treatment of asthma. It is a prodrug of terbutaline. Bambuterol causes smooth muscle relaxation, resulting in dilation of bronchial passages.

MATERIALS AND METHODS :

Chemicals and Drugs-

1. Bambuterol Hydrochloride IP
2. Purified Water

All drugs used during the experimental work are provided as gift sample by INDUJA Chemicals Pvt. Ltd. Sambhaji Nagar And other chemicals and reagent used are taken from Saraswati Institute of Pharmacy (CollegeLaboratory).

Apparatus-

1. Volumetric flask
2. Measuring Cylinder
3. Beaker
4. Spatula
5. Pipette
6. Mortar and pestle

EXPERIMENTAL

1. Selection of Common Solvent-

Purified Water was selected as common solvent for developing spectral characteristics of drug. The selection was made after checking the solubility of the drugs in different solvents.

2. Preparation of Standard Stock Solution-

a) Bambuterol Hydrochloride standard stock solution:(100µg/ml):

Accurately weighed 100mg of reference standard of Bambuterol Hydrochloride was transferred to 100ml volumetric flask dissolve in 100ml of water ; sonicated for 5min. Then volume was made upto the mark with water to obtain standard stock solution (100µg/ml) of drug. Sonicate for 10min. For the

preparation of working standard, suitable aliquot of stock solution were pipette out and volumes were made up to the mark with water so as to get concentration 1 µg/ml to 6µg/ml.

3. Selection of analytical wavelength:

For the estimation of the drug, wavelength maximum of Bambuterol Hydrochloride was determined and founded to be 264 nm (λ_{max}) respectively where there was no interference among the drug. Calibration curve was plotted between absorbance and is nominal concentration in the range of 1 to 6 Bambuterol hydrochloride at their respective maxima.

Selection of analytical concentration range:

Stock solutions of BAM were prepared by dissolving 100 mg of BAM dissolved in water and then the volume was adjusted to100 ml with purified water separately.

Stock solution of BAM were subsequently diluted with purified water to get 1,2,3,4,5,6 µg/ml respectively. Then the absorbance of these diluted solutions were measured at 264 nm (λ_{max}) for BAM by using double beam U. V. Spectrophotometer against a blank of water. Average of six replicates readings was taken and tabulated. Regression equation was derived from the slope of the curve Y= 0.251X0.0007; r^2 = 0.999 for BAM.

Table 3: Linearity study of BAM HCL

Sr. No.	Concentration (µg/mL)	Absorbance(nm)	Regression Data		
1.	1	0.053			
2.	2	0.104			
3.	3	0.157			
4.	4	0.215	m=0.251	C=0.00075	r^2=0.999
5.	5	0.265			
6.	6	0.315			

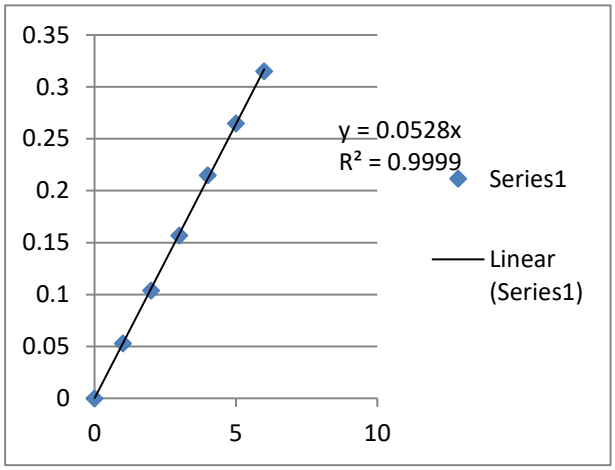

Fig 3: Calibration curve for BAM HCL:

Analysis of marketed formulation:

Twenty tablets were accurately weighed and crushed to get the fine powder. Powder equivalent to 10 mg of BAM was weighed and dissolved in water, sonicated for 20 min and filtered. Then different concentration of tablet sample were prepared by serial dilution technique and used for analysis.

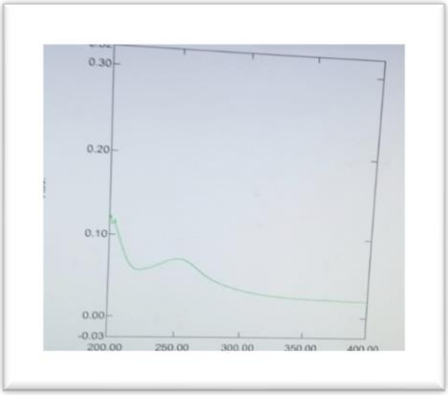

Fig 5: Overly spectrum of BAM HCL:

4. METHOD VALIDATION:

The method validation parameters Linearity, precision, accuracy, Repeatability, limit of detection and limit of quantification were checked as per ICH guidelines.

a) Linearity and Range

The Linearity for BAM were determined at six concentration levels, rangingfrom1-6µg/ml respectively using working standard.

b) Precision and accuracy

Precision of the method was evaluated by interday and intraday variation studies. Interaday studies, working solution of standard and sample were analyzed thrice in a day and percentage relative standard deviation (%RSD) was calculated. In the interday variation studies Working solution of standard and sample were analyzed on two consecutive days and percentage relativestandarddeviation(%RSD)wascalculated.thedataisreportedintable8and9

a) Limit of Detection and limit of Quantification

The **limit of detection (LOD)** is the smallest concentration of the analyte that gives the measurable response.LOD was calculate dosing the following formula and shown in Table 4.

$$LOD=3.3\ (\sigma /S)$$

Where
S=Slope of calibration curve,
σ=Standard deviations of the response.
The **limit of Quantification (LOQ)** is the smallest concentration of the anlayte, which gives are response that can be accurately quantified.LOQ was calculated using the following formula andshownTable12.

$$LOQ=10(\sigma/S)$$

Where
S= Slope of calibration cut,
σ=Standard deviation of the response.

b) Recovery study

To check accuracy of proposed method recovery were carried out at 80%, 100% and 120% of the test concentrations per ICH guideline.
To perform recovery studies at 100% tablet powder equivalent to 10mg of BAM HCL was weighed. To this 10mg of standard BAMHCL was added. From this powder an equivalent of 10 mg of BAM HCL were transferred to 100 ml of volumetric flask. The volume was made up to Mark with water and further dilution with mobile phase to obtain sample solutions.
Similarly for 80% of recovery studies 8 mg and 120% recovery 12 mg of standard Bambuterol hydrochloride where added respectively. The recovery studies where perform three times at each level the result of recovery study along with its statistical validation is given in table 10and11.

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RESULT AND DISCUSSION :

Method validation

The proposed method was validated by studying several parameters such as Linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

Linearity

Linearity range of BAM HCL was found to be 1to 6ug/ml. The results of Linearity studies are given table.5.

Table-5 Linear regression data for calibration curve of BAM HCL

Drug	**Linearity Range (µg/ml)**	**Slope**	**Intercept**	**Regression Coefficient**
BAM HCL	1-6	0.251	0.0139	0.999

Analysis of tablet formulation

Form analysis of tablet formulation content of bambuterol hydrochloride in tablet dosage form was calculated using simultaneous equation as an respectively.

Table 6. Analysis of tablet formulation

Sr.no.	**Lable claim(mg/tab)**	**Amount found (mg/tab)**	**%Of Label Claim**
	BAM HCL	**BAM HCL**	**BAM HCL**
1	10	10.00	101.78
2	10	9.98	99.63
3	10	9.98	99.63
4	10	9.98	99.63
5	10	10.00	99.60
6	10	10.02	100.40
Mean*			100.11
SD*			0.8745
RSD*			0.8738

*Denotes average of 6 determinations

Precision of method

The repeatability of sample application and measurement of peak area were express in item of%
R.S.D and found to be less than 2% the result of repeatability and precision studies are given in table 7,8,and 9

Table7: Repeatability data

Component	**%Mean***	**Standard Deviation***	**Relative Standard Deviation***
BAM HCL	99.88	0.9012	0.9034

***Denotes average of 6 determination

Table 8: Intra day precision data

Component	%Mean.	S.D.	%R.S.D.
BAM HCL	100.15	0.8410	0.8397

*Denote average of 6 determinations

Table9::Interday precision data

Component	%Mean.	S.D.	%R.S.D.
BAM HCL	100.25	0.8410	0.8397

*Denote average of 6 determinations

Recovery studies:

The recovery studies were performed at 80,100 and 120% of test concentration as per ICHguideline.the result of recovery studies along with its statistical validation are given in table 10and11.

Table10: Result of recovery study

Level of	Amount Present (mg/tab)	Amount Of pure Added(mg)	Total amount recovered (mg)	%Recovery
	BAM HCL	BAM HCL	BAM HCL	BAM HCL
80	10	8	18.3	100.18
	10	8	18.03	100.37
	10	8	18.23	102.57
100	10	10	20.96	99.63
	10	10	20.08	100.87
	10	10	20.08	100.87
120	10	12	22.01	100.15
	10	12	22.01	100.15
	10	12	22.01	100.15

Table11: statistical validation of recovery study

Level Of Recovery%	%Mean*		Standard Deviation*		Relative Standard Deviation*	
	BAM HCL	AMB HCL	BAM HCL	AMB HCL	BAM HCL	AMB HCL
80	101.04	100.16	1.3284	0.0850	1.3147	0.0848
100	100.46	100.20	0.7159	0.0981	0.7085	0.0979
120	99.36	100.36	1.7404	0.0963	0.7516	0.0961

*Denotes average of 3 determinations.

SUMMARY AND CONCLUSION :

Table 12: summary of validation parameters for dual wavelength method:

Parameters		BAMHCL
Linearity range (µg/ml)		1-6
Correlation coefficient(r^2)		0.999
Precision(RSD)	Intraday*	0.8397
Accuracy(%)	80%@	101.04
	100%@	100.46
	120%@	99.36

Repeatability (RSD)*	0.7403
LOD(µg/ml)	0.0355
LOQ(µg/ml)	0.1071

*Six Derermination, @Three determination, RSD-Relative Standard Deviation

CONCLUSION:

The method permits simple rapid and direct determination of BAM HCL and AMB HCL.in The result of analysis of two drugs from tablet formulation using method was found close to 100%, satndard deviation was satisfactorily low indicating accuracy and reproducibility of the method. Recovery studies were satisfactory which showed that the reisno interference of excipients.

The most striking feature of this method is its simplicity and rapidity ,non requiring–consuming sample preparation such extraction of solvents ,heating degassing which are generally needed for HPLC analysis .it is a new and novel method and can be employed for routine quality control analysis .the described method gives accurate and precise results for determination of BAM HCL and AMB HCL in tablets.

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